STUDY SPONSOR: CBI
STUDY TYPE:
TEST ITEM:
STUDY NUMBER:

INTRODUCTION

A study was performed to assess the irritation of the test item to the eye of the New Zealand White rabbit (General Study Plan 560-17).

This study was designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guidelines for the Testing of Chemicals No. 405 "Acute Eye Irritation/Corrosion" (adopted 02 October 2012)
- Method B5 Acute Toxicity (Eye Irritation) of Commission Regulation (EC) No. 440/2008

Initial considerations

Available information indicated that the test item had the potential to produce severe effects in a rabbit eye and to confirm this initial assessment, a Rabbit Enucleated Eye Test was performed prior to the *in vivo* test. The results are given in the Appendix and indicated that the test item was unlikely to cause severe ocular irritancy. The negative control data was shared with CBI CBI

Experimental Starting Date: 24 June 2014
Experimental Completion Date: 17 July 2014

Test item characterisation data are the responsibility of the Sponsor.

All raw data will be retained in Harlan Laboratories Ltd., Shardlow, UK archives.

METHOD

A single application of 0.1 mL of the test item was administered to the non-irrigated eye of two rabbits. Ocular reactions were recorded 1, 24, 48 and 72 hours after administration.

RESULTS

Individual and group mean scores for ocular irritation are given in Table 1 and Table 2.

A single application of the test item to the non-irrigated eye of two rabbits produced iridial inflammation and/or moderate conjunctival irritation. Both treated eyes appeared normal at the 72-Hour observation.

CONCLUSION

The test item produced a maximum group mean score of 10.5 and was classified as a mild irritant (Class 4 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification system.

This study was conducted in a facility operating to Good Laboratory Practice within the UK national GLP monitoring programme, but the study report has not been audited by the QA Unit. No formal claim of GLP compliance is made for this study. This report is an accurate record of the study and its outcome.

A. Pooles

Date: 6/10/14

A Pooles
Study Director

TABLES

Table 1 Individual Scores and Individual Total Scores for Ocular Irritation

Rabbit Number and Sex	74465 Female				74508 Female			
	IPR = 0				IPR = 0			
Time After Treatment	1 Hour	24 Hours	48 Hours	72 Hours	1 Hour	24 Hours	48 Hours	72 Hours
CORNEA								
E = Degree of Opacity	0	0	0	0	0	0	0	0
F = Area of Cornea Involved	0	0	0	0	0	0	0	0
Score (E x F) x 5	0	0	0	0	0	0	0	0
IRIS								
D	1	0	0	0	0	0	0	0
Score (D x 5)	5	0	0	0	0	0	0	0
CONJUNCTIVAE								
A = Redness	2	1	1	0	2	1	1	0
B = Chemosis	1	1	0	0	1	1	0	0
C = Discharge	1	0	0	0	1	0	0	0
Score (A + B + C) x 2	8	4	2	0	8	4	2	0
Total Score	13	4	2	0	8	4	2	0

IPR = Initial pain reaction

Table 2 Individual Total Scores and Group Mean Scores for Ocular Irritation

Rabbit Number and Sex	Individual Total Scores At:			
	1 Hour	24 Hours	48 Hours	72 Hours
74465 Female	13	4	2	0
74508 Female	8	4	2	0
Group Total	21	8	4	0
Group Mean Score	10.5	4.0	2.0	0.0

APPENDIX

Rabbit Enucleated Eye Test

Introduction

The ocular irritancy potential of the test item was assessed using the Rabbit Enucleated Eye Test. This method involved the application of the test item onto the cornea of the enucleated eye. The Rabbit Enucleated Eye Test is used as a first stage in the assessment of ocular irritancy potential. A negative ocular irritancy potential may require further investigation using an *in vivo* ocular irritation test. The test has undergone validation and has been shown to reliably detect substances that are negligible, or moderate to severe ocular irritants.

Methods

Five enucleated eyes, obtained from the New Zealand White strain of rabbit, were maintained at a temperature of 32 ±1.5 °C within the superfusion apparatus. 0.1 mL was applied onto the cornea of each of three enucleated eyes. The direct effect of the test item on the cornea was assessed by evaluation of corneal thickness, corneal opacity, alteration of corneal epithelium and fluorescein uptake, throughout the duration of the test. The data for all endpoints was assessed and an estimate of ocular irritancy potential made.

A further two enucleated eyes remained untreated for control purposes. The negative control data was shared with CBI

Results

	Corneal Opacity							
	Observation Period (minutes post dosing)							
	60		120		180		240	
	Cldy	Area	Cldy	Area	Cldy	Area	Cldy	Area
Test Eyes	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Control Eyes*	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Cldy = Corneal cloudiness
* = Control data shared with Harlan Laboratories Ltd. Study number 41401640

	Corneal Epithelium Condition			
	Observation Period (minutes post dosing)			
	60	120	180	240
Test Eyes	Normal	Normal	Normal	Normal
	Normal	Normal	Normal	Normal
	Normal	Normal	Normal	Normal
Control Eyes*	Normal	Normal	Normal	Normal
	Normal	Normal	Normal	Normal

	Fluorescein Uptake (240 minutes post dosing)				
	Test Eyes			Control Eyes*	
Int	0	0	0	0	0
Area	0	0	0	0	0

	Corneal Swelling (%) (minutes post dosing)		
	60	120	240
Test Eyes[a]	12.1	8.3	2.2
Control Eyes*[b]	10.3	8.8	6.3

Conclusion

Following assessment of the data for all endpoints the test item was considered unlikely to have the potential to cause severe ocular irritancy *in vivo*.

* = Control data shared with CBI
Int = intensity of fluorescein uptake
a = for each time point the corneal swelling recorded is the mean of three eyes
b = for each time point the corneal swelling recorded is the mean of two eyes